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ON THE MIGRATING COURSE OF ASCARID LARVAE IN THE BODY OF THE HOST

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In my previous paper (Yoshida, 1919) I have reported that the larvae hatched out in the intestine of a feeding animal, migrate into the liver, lungs and trachea successively and they again pass down the alimentary canal to the intestine, being finally evacuated with the feces sooner or later. The exact course of migration, however, remained to be reported. Since April of this year I have devoted myself to decide experimentally the general course of migration by the larvae in the body of the host. For this purpose, two sets of experiments have been made, (1) experiments injecting larvae into the body of the animal and (2) feeding experiments with the ripe eggs. In the first set of experiments, the migratory power of the larvae was examined chiefly and in the second set the natural and actual course of migration was observed. Both sets of experiments gave successful results from which I have learned the general course of migration from the intestine to the lung.

Before proceeding to describe the experiments, it seems necessary to mention here briefly my methods for injecting larvae and finding them in various organs and tissues. The larvae in the pleural or abdominal cavity are easily collected by centrifuging the fluid in the cavity. As washing fluid 10 or 20 c.c. of physiological salt solution was usually used.

For collecting larvae in any organ, the tissue, such as liver, lungs, etc., is gently brayed in the earthenware mortar with a small quantity of physiological salt solution, the particles of the organ are mixed with 10 or 20 c.c. of physiological salt solution and then filtered through a wire net or gauze of the definite size of mesh to remove the coarse particles. The larvae finally are easily collected by centrifuging the filtered fluid. The coarse particles are to be repeatedly brayed and filtered as before until larvae are no longer present. The larvae thus collected from any organ or tissue are injected with 2 or 5 c.c. of physiological salt solution. Injection was made into the subcutaneous layer, abdominal or pleural cavity of guinea-pig.

Injection Experiments

As to the migrating power of the ascarid larvae, results of two injecting experiments were reported in my previous paper. Since then numerous experiments were tried by injecting the larvae into the abdominal or pleural cavity, or into the subcutaneous layer of the animal.

Exp. 1.—At 1 p. m., on June 6, larvae collected from the liver of a guinea-pig, killed seventy-five hours after feeding with ripe eggs, were injected into the abdominal cavity of a guinea-pig A. On the next morning after, about twenty hours, the animal was found dead. In the pleural cavity were found two living larvae, but none in the lungs. In the abdominal cavity numerous dead larvae were present and a few in the liver.

Exp. 2.—At 1 p. m., on June 6, larvae from the liver of the same animal as in the preceding experiment were injected into the pleural cavity of two guinea-pigs, C and D. On the next morning the animal C was found dead after twenty hours; in the pleural cavity numerous dead larvae were present and a few living ones in the lungs.

Exp. 3.—The animal D was also found dead in the same morning, the result of examination was the same as in the preceding case.

Exp. 4.—At 8 a. m., on June 4, larvae from the lungs of a guinea-pig, killed sixty-five hours after feeding, were injected into the abdominal cavity of a guinea-pig B. On the next morning the animal was found dead after twenty-four hours. The lungs and liver were infected, many dead larvae being in the abdominal cavity and a few living in the pleural.

Exp. 5.—At 9 a. m., on June 10, larvae from the liver of an animal, killed sixty-six hours after feeding, were injected into the abdominal cavity of a guinea-pig B. The animal was found dead on the next morning after twenty-four hours. Numerous dead and living larvae were found in the abdominal cavity, a few in the liver, five in a piece of the lung and none in the pleural cavity.

Exp. 6.—At 2 p. m., on June 10, the larvae from the liver of the guinea-pig, killed seventy-one hours after feeding, were injected into the abdominal cavity of two guinea-pigs C and D. In the evening of the next day, the animal C was found dead after about twenty-seven hours. On dissection it was discovered that large quantities of the injected matter were accumulated between the skin and the underlying muscle layer. (It was generally observed that the injected particles of the lung or liver with the larvae were gathered into masses in the abdominal or pleural cavity into which they were injected.) In the masses of the injected particles of the liver, larvae were present and dead larvae were found in the abdominal cavity, a few living ones in the liver; they were relatively numerous in the lungs while none were in the pleural cavity.

Exp. 7.—Animal D of the preceding experiment died in the same evening; the result of examination was quite the same as C.

Exp. 8.—A 8 a. m., on June 3, larvae from the liver of a guinea-pig, killed forty-eight hours after feeding, were injected into the abdominal cavity of two guinea-pigs C and D. Animal C was killed at 8 a. m. on the 5th, after forty-one hours. An abundance of larvae were present in the lungs and a few in the liver, while none were found in the pleural and abdominal cavity.

Exp. 9.—Animal D was killed at 3 p. m. on the 5th, after forty-three hours. Numerous larvae were found in the lungs, but none in the pleural cavity and a few in the liver.

Exp. 10.—At 9 a. m., on June 10, larvae from the lungs of a guinea-pig, killed sixty-six hours after feeding, were injected into the abdominal cavity of guinea-pig A. The animal was killed at 4 p. m. on the 12th, after fifty-five hours. A few larvae were present in the lungs and the other organs were unexamined.

Exp. 11.—At 2 p. m., on June 4, larvae from the liver of a guinea-pig, killed after seventy-one hours after feeding, were injected into the abdominal cavity. The animal was killed at 8 a. m. on the 7th, after sixty-six hours. I found two specimens of larvae in the pleural cavity, four in the abdominal cavity, a few in the lungs and a very few in the liver.

Exp. 12.—At 2 p. m., on the 4th, larvae from the lungs of the same guinea-pig were injected into the abdominal cavity of animal B. It was killed at 10 a. m. on the 10th, after ninety-two hours. On the dissection it was found that the injected materials were all introduced into between the skin and the underlying muscle layer. In the accumulated masses of the injected materials larvae were present and a few larvae in the liver as well as in the abdominal cavity. None in the lungs and pleural cavity.

From the observation in this experiment we may easily believe that the larvae migrate into the body cavity through the muscular wall of abdomen.

Exp. 13.—At 11 a. m., on May 10, larvae from the lungs of the guinea-pig, killed after ninety-one hours from feeding, were injected into the abdominal cavity. The animal was killed at 8 a. m., on the 15th, after 117 hours. A few larvae were found in the lungs, but none in the liver.

Exp. 14.—At 11 a. m., on May 11, larvae from the lungs of the guinea-pig, killed after ninety-one hours from feeding, were injected into the abdominal cavity. It was killed at 9 a. m. on the 16th, after 118 hours. The lungs were tolerably hemorrhaged and the larvae were present.

Exp. 15.—At 10 a. m., on May 4, the larvae from the liver of a guinea-pig killed after sixty-nine hours from feeding, were smeared over the abdominal skin from which the hair was removed by cutting closely and shaving. The animal was fixed on the holder during about four hours after operation, restricting the violent movement in order to prevent the loss of larvae and to avoid larvae being taken into the mouth of the animal. After 5 p. m. the animal was put in the separate cage and the smeared part of abdomen was closely covered by the cloth. In the next morning the animal was put in the same cage as others. At noon on the 11th larvae from the liver of a guinea-pig killed after 118 hours, were smeared on the nape of the neck. The animal was killed at 9 a. m. on the 17th, after thirteen and six days from the first and the second smearing, respectively. Five specimens of larvae were obtained from a piece of the lung, two in a part of the large intestine and none in the liver. Almost all these larvae were fully developed, being about 1.8 mm. long. This experiment shows evidently that larvae on the skin may pierce through the body wall of host. It also proves how important and necessary the lungs are for the further development of the ascarid larvae.

From observations in above experiments it may be easily recognized that the larvae injected into the pleural cavity penetrate into the lungs directly, as Experiments 2 and 3 show, and those injected into the abdominal cavity penetrate into the liver or pierce the diaphragm to enter the pleural cavity and thence to reach the lungs. These facts show great power of the larvae in boring through various tissues, as skin, muscle, tendon and several parenchymatous tissues.

Some larvae in the abdominal cavity might have reached the lungs passing through the liver and heart by the way of a blood vessel, but the majority of them are considered to have proceeded directly to the lungs piercing through the diaphragm. It is reasonable to think so from the nature of the larvae, which have marked power in boring

TABLE 1

No.	Date Injected	Injecting Place	Age of Larvae	Date Killed	Duration of Infestation	Pleural Cavity	Lungs	Abdominal Cavity	Liver
1	1 p. m. June 6 A	Abdominal cavity	75 hours from liver	Died morning June 7	< 20 hr.	3	None	Many dead larvae	Present
2	1 p. m. June 6 C	Pleural cavity	75 hours from liver	Died morning June 7	< 20 hr.	Present	A few		
3	1 p. m. June 6 D	Pleural cavity	75 hours from liver	Died morning June 7	< 20 hr.	Present	A few		
4	8 a. m. June 4 B	Abdominal cavity	65 hours from lung	Died morning June 5	< 24 hr.	A few	Present	Dead larvae	Present
5	9 a. m. June 10 B	Abdominal cavity	66 hours from liver	Died morning June 11	< 24 hr.	None	5	Present	A few (? in a piece)
6	2 p. m. June 10 C	Abdominal cavity	71 hours from liver	Died evening June 11	< 27 hr.	None	Many	Many	A few
7	2 p. m. June 10 D	Abdominal cavity	71 hours from liver	Died evening June 11	< 27 hr.	None	Many	Many	A few
8	3 p. m. June 3 C	Abdominal cavity	48 hours from liver	Killed 8 a. m. June 5	41 hr.	A few	Many	None	A few
9	3 p. m. June 3 D	Abdominal cavity	48 hours from liver	Killed 3 p. m. June 5	48 hr.	None	Many	A few	A few
10	9 a. m. June 10 A	Abdominal cavity	66 hours from liver	Killed 4 p. m. June 12	55 hr.	None	A few	None	None
11	2 p. m. June 4 C	Abdominal cavity	71 hours from liver	Killed 8 a. m. June 7	66 hr.	2	A few	4	A few
12	2 p. m. June 4 D	Subcutaneous	71 hours from lung	Killed 10 a. m. June 8	92 hr.	None	None	A few	A few
13	11 a. m. May 10	Abdominal cavity	91 hours from lung	Killed 8 a. m. May 15	117 hr.	......	A few	......	None
14	11 a. m. May 11	Abdominal cavity	91 hours from lung	Killed 9 a. m. May 16	118 hr.	......	Present		
15	1 p. m. May 4, noon May 11	Smeared on abdominal and nape	69 hours and 91 hours	Killed 9 a. m. May 17	13 or 6 days	......	5 and 2 in large intestine		

tissue. Moreover, the appearance of larvae in the pleural cavity clearly proves their direct migration from the abdominal cavity.

FEEDING EXPERIMENTS

Such power of larvae in boring through the tissues makes it reasonable to think that larvae hatched in the intestine may pierce through the intestinal wall to enter the abdominal cavity. This is also surely and actually confirmed by the following feeding experiments.

Feeding experiments were chiefly intended to examine whether the larvae just escaped from the eggshell in the intestine of host may pierce through the intestinal wall or not, and to trace the subsequent course of migration made by the larvae.

Exp. 16.—At noon on June 14, two guinea-pigs A and B were fed with the ripe eggs of sixty-one days old, and the A was killed at 8 a. m. on the next day after twenty hours. The lungs were slightly hemorrhaged, spotted with bloody color here and there, contained numerous larvae of Ascaris. Five specimens of larvae were obtained from the pleural cavity, most of them were surrounded by a group or mass of white blood corpuscles and histiocytes. Many larvae were present in the liver as well as in the abdominal cavity, and also three in the spleen, four in the pancreas and two in the left kidney. All larvae found in these organs were quite as young as those just hatched out in the intestine, in size and in organization of body, measuring from 0.2 to 0.24 mm. in length. Thus the larvae found in the abdominal cavity and in other organs may surely be considered to have pierced through the intestinal wall. This was repeatedly proved by the following experiments.

Exp. 17.—The animal B of the preceding experiment was killed at noon on the 15th, after twenty-four hours. The lungs and liver were infected, three larvae in the pleural cavity, two in the spleen, one in the right kidney, three in the pancreas and many in the abdominal cavity. Larvae were all in the same size as in the preceding case.

Exp. 18.—At 3 p. m., on June 12, two guinea-pigs A and B were fed with ripe eggs sixty-four days old. A was killed at 8 a. m. on the 14th, after forty-one hours. The lungs and liver were heavily infected. Five specimens of larvae in the pleural cavity, many in the abdominal cavity, four in the kidneys, one in the pancreas and none in the spleen.

Exp. 19.—Animal B of Experiment 18 was killed at 10 a. m., on the 14th, after forty-three hours. Result of examination was the same as in the case of A, but two larvae were in the pancreas and three in the kidneys.

Exp. 20.—At 3 p. m., on June 1, three guinea-pigs were fed with the ripe eggs of fifty-two (to A and B) and of fifty-three (to C) days old. C was killed at 3 p. m. on the 3rd, after forty-eight hours. The lungs and liver were infected. Larvae were found in the spleen, in the pleural and abdominal cavity. The pancreas and kidneys were unexamined.

Exp. 21.—Animal B of preceding experiment was killed at 8 a. m. on the 4th, after sixty-five hours. The lungs and liver were infected, twelve larvae being in the abdominal cavity, two in the kidneys, four in the pancreas, while none were in the spleen or in the pleural cavity.

Exp. 22.—At 3 p. m., on June 7, two guinea-pigs (A and B) were fed with the mature eggs, fifty-nine days old; B was killed at 9 a. m. on the 10th, after sixty-six hours. The lungs and liver were infected, the former organ being heavily hemorrhaged. Other organs were unexamined, but the spleen with that of A contained nine specimens of larvae.

Exp. 23.—Animal A of Exp. 22 was killed at 2 p. m. on that day, after seventy-one hours. The lungs and liver were in quite the same state of infection as in the preceding case. Eleven larvae were obtained from the pleural cavity, six of them were in the state of free movement, and the remaining five surrounded by the masses of white blood corpuscles and histiocytes. Eight were in the abdominal cavity, four in the kidneys and two in the pancreas.

Exp. 24.—Animal A of Exp. 20 was killed at 2 a. m. on the 4th, after seventy-one hours. The lungs and liver were heavily infected, two specimens of larvae were present in the pleural cavity, one in the spleen, three in the kidneys and many in the abdominal cavity, but none in the pancreas.

Exp. 25.—At 10 a. m. on June 3, two guinea-pigs A and B were fed with ripe eggs fifty-five days old, they were killed at 1 p. m. on the 6th, after seventy-five hours. In A the lungs and liver were infected, four larvae in the pleural cavity, eight in the abdominal cavity, two in the spleen, one in the pancreas and none in the kidneys. In B the lungs and liver were in the same state as in the case A, a few larvae in the abdominal cavity, two in the kidney, but none in the pleural cavity, spleen and pancreas.

TABLE 2

No.	Feeding Date	Age of Eggs, Days	Killing Date	Hours Passed	Pleural Cavity	Lungs	Ab-dom. Cavity	Liver	Spleen	Pan-creas	Kidney
1	Noon June 14 A	61	8 a. m. June 15	20	5	Bloody spot, many larvae	Many	Many	3	4	2 (left)
2	Noon June 14 B	61	Noon June 15	24	3	Slightly blooded present	Many	Present	2	3	1 (rt.)
3	3 p. m. June 12 A	64	8 a. m. June 14	41	5	Many	Many	Many	None	1	4
4	3 p. m. June 12 B	64	10 a. m. June 14	43	Present	Many	Many	Many	None	2	3
5	3 p. m. June 1 C	53	3 p. m. June 4	48	Present	Present	Present	Present	Present		
6	3 p. m. June 1 B	52	8 a. m. June 4	65	None	Present	12	Present	None	4	2
7	3 p. m. June 7 B	59	9 a. m. June 10	66	......	Heavily blooded present	......	Present	9		
8	3 p. m. June 7 A	59	2 p. m. June 10	71	11	Heavily blooded present	8	Present		2	4
9	3 p. m. June 1 A	52	2 p. m. June 4	71	2	Heavily blooded present	Many	Present	1	None	3
10	10 a. m. June 3 A	55	1 p. m. June 6	75	4	Heavily blooded present	8	Present	2	1	None
11	10 a. m. June 3 B	55	1 p. m. June 6	75	None	Heavily blooded present	Present	Present	None	2	None

Results of these feeding experiments give the facts accurately in the course of the migration carried out by the ascarid larvae in the body of host, as was surely decided in combination with the result of above injecting experiments.

Larvae hatched out in the intestine immediately proceed to the abdominal cavity piercing through the intestinal wall. Larvae in the cavity may wander everywhere freely, that is, some to the liver, spleen, pancreas or kidneys in the cavity and others piercing the diaphragm to enter the pleural cavity, finally penetrating into the lung from its surface. Thus it is believed that the larvae migrate in every direction, boring through various organs or tissues by means of their own power of piercing, but not by the way of blood vessels.

As to the course of larvae migrating from the intestine to the lung F. H. Stewart supposed two ways and said: "1. Boring through the wall of the stomach or intestine the larva enters a mesenteric venule and is carried to the liver. Thence they pass in the hepatic vein to the heart and by the pulmonary artery to the lung. 2. The larva after hatching in the stomach or duodenum travels by the bile duct and through the degenerated liver tissues and reaching a hepatic venule continues its course as in the first case."

Stewart's supposition might be true, but it would be an accidental case and not the sole or general course of migration, I think. If a blood vessel is the only way by which the larvae migrate in the body of host, the appearance of larvae in the pleural cavity is very difficult to explain.

It was formerly considered by Stewart as well as myself that larvae migrate successively from the intestine to the lung, passing through the liver, in consequence of which the larvae appear in the lungs later than in the liver. From this belief it was easy to think the larvae travel in the blood vessel from the liver to the lung, passing through the heart. But the belief in this successive migration of larvae has been radically destroyed by my above experiments.

The larvae in the abdominal cavity may easily wander about everywhere, and penetrate not only into various organs or tissues in the cavity, but into the lungs, piercing through the diaphragm. Thus the liver, with several other organs in the abdominal cavity, is not a necessary and important organ to be passed for the larvae to reach the lung, as we formerly considered. Larvae penetrating into the liver might be considered to travel to the lungs by the way of a blood vessel, but it is not the general way for larvae to reach the organ. Hence I am inclined to believe that the larvae migrate from the intestine to the lung by their strong power of boring through the various organs or tissues, but not by the way of a blood vessel.

The general and important course of migration by the ascarid larvae in the body of host may be as follows: The ascarid larvae escape from the eggshell in the intestine of host and proceed to the abdominal cavity by boring through the wall of intestine. Thence they pierce the diaphragm to enter the pleural cavity, finally penetrating into the lungs from their surface. It might be considered as an additional and mere accidental course of migration that the larvae in the abdominal cavity penetrate into the liver, thence they are carried to the lungs by the way of blood vessels passing through the heart. This belief in the course of migration of the larvae is strengthened by histological study of the infected organs and tissues. It shows that almost all the larvae in the lungs and liver are not found in the blood vessels but in other tissues. Details on this side, however, will be reported in another paper.

Furthermore, the lungs are the only necessary and important organ to be passed by the larvae in the course of their development. In this organ the larvae stay longer than in any other, and they develop rapidly there as completely as possible, reaching a length of about 1.7 to 2.0 mm. Larvae in the lungs, several days after feeding, are generally much larger than those in any other organ. This fact was repeatedly observed in recent experiments. The results of some experiments on this subject are given in Table 3.

TABLE 3

Duration of Infestation	Length of Worm in						
	Lungs	Pleural Cavity	Abdominal Cavity	Liver	Spleen	Pancreas	Kidney
162 hrs.	0.3 -0.57 0.95-1.0	0.28 0.48	0.28 0.48	0.28 0.48			
166 hrs.	0.4 -0.51 0.85-1.33	0.28 0.46	....	0.4	0.28	0.28 0.44	0.28
250 hrs.	1.8 2.0	0.3	0.28				

The larvae in the lungs continue their development and migrate to the mouth cavity through the trachea, again passing down the alimentary canal to the intestine of the host.

The occurrence of larvae in the spleen was first mistakenly reported by Stewart in his first paper, and corrected in his second paper. Afterward B. H. Ransom and W. D. Foster recognized the presence of larvae in the organ. I have frequently found the larvae not only in the spleen but in the pancreas and kidneys. The occurrence of larvae in these organs is easily explained by their power in boring through tissues. It should be, therefore, clear that the larvae might penetrate into other organs or tissues such as ovarium, adrenal body, etc., which as yet I have not examined. All these organs and tissues are accidentlly or temporarily visited by the larvae and in them they are not able to complete or continue development. Larvae in the liver, however, might be carried in the hepatic vein to the heart and consequently by the pulmonary artery to the lungs, where they can develop further. The fate of larvae in organs or tissues other than the lung is not yet traced accurately, experiments on this problem being under way. From the results of some experiments, however, I have learned the following facts: After several days (five to ten) after feeding, few larvae or none were found in these organs, but they were more or less present in the abdominal and pleural cavity. The more time passed the more the larvae decreased in number in these organs while the more they increased in the lung. The larvae in these organs were much smaller than those in the lung, and some of the former were dead or decomposed in structure. So great variation is found in size of larvae in the lung that it cannot be caused by the individual difference only. The larger specimens were three or four times as large as the smaller one, which was in the same size as those in the liver and other organs or tissues. This great variation is probably due to a different invasion of larvae. It is easy to see that the rapid growth of larvae in the lungs may cause great difference in size according to difference in time of invasion.

From these facts we may infer that some larvae in these organs may go back in the abdominal cavity and proceed to the pleural cavity to invade the lungs, the important place for their further development, and those remaining probably perish in these organs. At any rate, the details of larval fate in these organs, except the lung, should be left untouched here.

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